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RESEARCH ARTICLE

Expression pattern and function analyses of the MADS thranscription factor genes in wheat (*Triticum aestivum* L.) under phosphorus-starvation condition

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Abstract

MADS-box (MADS) transcription factors (TFs) act as one of the largest TF families in plants. The members in this family play fundamental roles in almost every developmental process as well as involve plant responses to biotic and abiotic stresses. In this study, 54 of MADS genes in wheat, including 31 released publicly and 23 deposited as tentative consensus (TC) into GenBank database, were subjected to analyses of molecular characterization, expression pattern, and function under contrasting phosphate (Pi)-supply conditions. The 31 released MADS genes share cDNA full lengths of 683 to 1 297 bp, encoding amino acids of 170 to 274 aa that possess molecular weights of 19.21 to 31.33 kDa and isoelectric points of 5.74 to 9.63. Phylogenetic analysis categoried these wheat MADS genes into four subgroups containing 11, 5, 10, and 4 members, respectively. Under Pi sufficiency, the MADS genes showed drastically varied transcripts and they were categoried into expression groups of high, medium, low, and very low, respectively. Among them, several ones were differentially expressed under Pi deprivation, including that five were upregulated (*TaMADS51*, *TaMADS4*, *TaMADS5*, *TaMADS6*, and *TaMADS18*) and four were downregulated (*TaMADAGL17*, *TaMADAGL2*, *TaMADWM31C*, and *TaMADS;14*). qPCR analyses confirmed their expression patterns in responding to the Pi-starvation stress. *TaMADS51*, one of the upregulated genes by Pi deprivation, was subjected to the functional analysis in mediating plant tolerance to the Pi-starvation stress. The transgenic tobocco plants overexpressing *TaMADS51* exhibited much more improved growth features, drymass, Pi acquisition, and photosynthetic parameters as well as antioxidant enzymatic activities under Pi deprivation than wild type. These results indicate that distinct MADS genes are transcriptional response to Pi deprivation and play critical roles in mediating plant tolerance to this stressor through regulating downstream Pi-responsive genes.

Keywords: wheat (*Triticum aestivum* L.), MADS type transcription factor, Pi deprivation, expression, gene function

1. Introduction

The MADS-box (MADS) proteins are important transcription factors (TFs) that are typified by MADS, a highly conserved DNA-binding domain located at N terminus. Thus far, the MADS family members are identified and charaterized in various plants, such as those of *Arabidopsis* (Parenicova *et al*. 2003), tomato (Hileman *et al*. 2006), rice (Arora *et al*.

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2007), and maize (Zhao *et al*. 2010). Based on phylogenetic analyses, plant MADS genes are categoried into two large lineages, types I and II that were generated by gene duplication occurred before the divergence of plants, fungi and animals (Nam *et al*. 2004). The type I MADS proteins generally harbor just one MAD box and are grouped into subclades of Mα, Mβ, and Mγ according to the sequences of MAD box and other additional motifs (De Bodt *et al*. 2003; Nam *et al*. 2004). In contrast, aside from the MAD box, the type II MADS proteins also share other conserved domains, such as those of K, I and C (Nam *et al*. 2004; Kaufmann *et al*. 2005), which have probably evolved the duplication of ancestral MIKC^C gene located in the Keratin-like region (Nam *et al*. 2004).

Plant MADS-type TFs play fundamental roles in almost every developmental process. Early studies revealed that *AGAMOUS* (*AG*) and *DEFICIENS* (*DEF*), two MADS genes from *A. thaliana* and *Antirrhinum majus*, respectively, act as crucial regulators in controlling the floral organ identity (Schwarz-Sommer *et al*. 1990; Yanofsky *et al*. 1990). In the past decade, a large set of plant MADS genes has been demonstrated to function essentially in diverse biological processes, such as those of root architecture establishment, gametophyte differentiation, fruit ripening, flowering time regulation, and reproductive organ development (Smaczniak *et al*. 2012). For example, five *Arabidopsis* MADS genes (i.e., *AGL30*, *AGL65*, *AGL66*, *AGL94*, and *AGL104*) are limited to express in pollen and act as critical components in the pollen development by repressing or activating expression of the immature pollen-related genes (Verelst *et al*. 2007a, b), indicating that the MADS TFs in plants involve many growth and developmental processes through transcriptional regulation of the distinct downstream genes.

Recently, plant MADS TFs have also been found to involve plant responses and tolerance to biotic and abiotic stresses. In rice, among the differentially expressed genes induced by inoculating *Magnaporthe oryzae* strain PLP-1, a set of them was identified to encode MADS proteins that seem to associate with the plant defence signaling through interaction with the incompatible host-pathogen (Gupta *et al*. 2012). In *Arabidopsis*, affymetrix global gene chip analyses revealed that several MADS genes are response to phosphate (Pi) deprivation (Misson *et al*. 2005). However, the functions of these MADS genes in mediating plant adaptation to the Pi-starvation stress are still largely unknown and needed to be further investigated.

Phosphorus (P) is one of the essential inorganic nutrients for plant growth, development and crop production. Large P fertilizer has been input in the arable field worldwide for a long time to reach the goal of high yield. However, a majority of P applied to soil is immobilized and becomes unavailable for plants (Vance *et al*. 2003), resulting in detrimental effect on environment as well as increasingly depletion on the non-renewable phosphate rock (Cordell *et al*. 2009). Elucidation of the global gene expression profile under Pi deprivation and identification of Pi signaling tansduction-associated genes in wheat as well as other crops could provide novel insights into understanding the molecular mechanism that plants tolerate the low-Pi stress and can direct the generation of crop cultivars with improved P use efficiency. In this study, 54 of MADS family genes in wheat were subjected to analyses of molecular characterization, global expression pattern and function under the contrasting Pi conditions. Our findings provide novel insights into the plant reponses and tolerance to Pi deprivation through the MADS-mediated pathways in wheat.

2. Materials and methods

2.1. Plant growth and stress treatments

Seedlings of Chinese Spring, a cultivar used extensively in wheat genetic and physiological studies, were cultured in an Murashige and Skoog (MS) nutrient solution as described by Sun *et al*. (2012). At the 3rd leaf-expansion stage, the seedlings were separately divided into two aliquots which were hydroponically cultured in normal MS (sufficient P, 1.2 mmol L^{-1} Pi) and modified MS with reduced Pi (12 μmol L^{-1} Pi), respectively. At 0 h (normal MS), and 1, 6 and 24 h after Pi deprivation, root tips of the seedlings were sampled to prepare probes for the microarray analysis and used as templates in the qPCR analysis that validated the expression patterns of the differentially expressed genes identified in microarray analyses.

2.2. Obtainment of the wheat MADS TF genes

The wheat MADS TF genes characterized in this study were derived from the probe sets arrayed in a wheat gene chip (Wheat Gene Expression Microarray GeneChip, Agilent Technologies) that represents 4×44K genes currently released in the GenBank database. Based on manual search, 54 genes categoried into the MADS TF family were identified in the chip. Of these, 31 are released in the National Center for Biotechnology Information (NCBI) GenBank and 23 are tentative consensus (TC) deposited in the wheat gene indice (DFCI) database. In this study, the MADS genes from the GenBank were named as those that they were submitted originally whereas the MADS genes from the DFCI databse were arbitrarily designated as names of *TaMAD;1* to *TaMAD;23*. The information such as names, GenBank acession numbers and probe set identities of these wheat

MADS genes is listed in Appendix A.

2.3. Characterization analysis of the wheat MADS genes

The molecular characterization of the wheat MADS genes derived from the NCBI GenBank was determined based on bioinformatic tools. The translated amino acids of the MADS genes were predicted by an online tool referred to protein translation from DNA provided in ExPasy (www.expasy.org). Molecular weight (MW) and isoeletric point (pI) of the translated MADS proteins were calculated by the DNAStar software. The DNA binding domain and the conserved MAD box of the MADS proteins were determined based on BLASTp analyses. The phylogenetic tree covering all of the wheat MADS TF genes was constructed by using a MegAlign algorithm program supplemented in the DNAStar software based on a distance method by comparing 1 000 bootstrap replicates.

2.4. Microarray analysis

A commercialized wheat gene chip (Agilent Technologies, USA) was selected to perform the microarray analyses on which to elucidate the global gene expression patterns upon Pi deprivation in wheat. Hybridization probes used for the microarray analyses were synthesized separately from the total RNA of root tips sampled at 0 h (sufficient-Pi, control) as well as 1, 6 and 24 h after Pi deprivation. Total RNA was extracted using TRIzol reagent (Invitrogen, USA) by following the manufacturer's instructions. Before synthesis of cDNA, the total RNA was treated with DNase to avoid the contamination by genomic DNA. cDNA labeling, prehybridization, hybridization, microarray slide scanning, and expression data normalization in the microarray analyses were all performed by a biotechnology service company (Phalanx Biotech Group, Taiwan of China). Three hybridization replicates for each probe set in the gene chip were conducted in order to yield reproducible results.

2.5. Analysis of the expression patterns of the wheat MADS genes

Expression levels of the 54 wheat MADS genes under two contrasting Pi conditions were determined based on the microarray analysis results. The MADS genes were classified into various expression groups according to the expression intensities under Pi sufficiency, including groups of high, medium, low and very low. Of these, the high group possessed intensity reads over 1 000 whereas the groups of medium, low, and very low displayed intensity reads from 100 to 1 000, 10 to 100 and below 10, respectively. According to $\log_2$ ratios between the intensity reads under Pi sufficiency and those at each time point under Pi deprivation, the MADS genes with differentially expressed patterns were identified. We arbitrarily termed the genes as differential ones as they possess $\log_2$ ratios over 1 or below –1 by following the widely adopted criterion in defining the differentially expressed genes in microarray analysis.

2.6. Analysis of the temporal and spatial expression patterns of the differentially expressed MADS genes

Plants grown in pots were treated by different Pi levels (sufficient- and deficient-Pi) and were used to analyze the temporal and spatial expression patterns of the differentially expressed MADS genes identified in the microarray analysis. Briefly, plants of Chinese Spring were cultured in pots filled with vermiculite in a growth room and were treated by two Pi treatments (sufficient-Pi, 1.2 mmol L^{-1}, and deficient-Pi, 100 μmol L^{-1}) by regularly providing MS solutions containing above Pi concentrations. At growth stages of 3rd-leaf expansion, jointing, booting, and flowering, various tissues of plants such as roots, leaves and young spikes (only at stages of booting and flowering) were sampled. Expression levels of *TaMADS51*, a significantly upregulated gene and *TaMADAGL2*, *TaMADWM31C* and *TaMADWM32B*, three significantly downregulated genes, were detected in above tissues under two Pi treatments based on qPCR analyses as described by Guo *et al*. (2013), in which a constitutively expressed gene in wheat named *Tatubulin* was used as internal standard. Primers for amplification of the MADS genes and *Tatubulin* are listed in Appendix B.

2.7. Trangenic analysis of *TaMADS51* in mediationg plant tolerance to Pi deprivation

TaMADS51 (GenBank acession number AB007506), a differentially upregulated gene upon Pi deprivation, was selected to further analyze the function in mediating plant tolerance to Pi deprivation based on genetic transformation analysis. The homologous partners of *TaMADS51* in plant species were obtained by BLASTn sarch analysis against the GenBank database using its cDNA as a query. Phylogenetic distance of *TaMADS51* and its homologs was calculated by Megalign alignment analysis as mentioned proviously.

The encoding frame of *TaMADS51* was obtained by RT-PCR using the deficient-Pi treated roots of Chinese Spring as templates and with specific primers (Appendix B). The confirmed sequencing products of *TaMADS51* were then integrated into binary vector pCAMBIA3301 at the position of downstream CaMV35S promoter. Binary plasmids were

then transformed into an *Agrobacterium tumefaciens* strain EHA105 and further genetically transformed tobacco (*cv*. Wisconsin 38) as described by Sun *et al*. (2012).

Nine independent transgenic tobacco lines generated were subjected to detection of the *TaMADS51* expression levels by qPCR as described by Guo *et al*. (2013) using the specific primers. The transgenic and wild type (WT) seedlings were hydroponically cultured in normal MS solution to the 3rd-leaf expansion stage, and then were treated by Pi deprivation (20 μmol L^{-1} Pi) for 24 h before sampled. Extraction of the total RNA, cDNA synthesis and qPCR were performed similar to those in detection of the temporal and spatial expression levels of *TaMADS51* as aforementioned, using tobacco *Nttubulin* as internal standard (Appendix B).

Lines 3 and 8, two transgenic lines with strong expressions of *TaMADS51* together with WT, were subjected to investigation of the function of this MADS gene in mediating plant tolerance to Pi deprivation, after detection of the target gene copies in their genome by following the conventional approach. Briefly, the transgenic and WT seedlings were cultured hydroponically in two Pi levels (sufficient-Pi, 1.2 mmol L^{-1}, and deficient-Pi, 50 μmol L^{-1}) by regularly providing MS solutions containing above Pi concentrations. During the process, the solutions were air circulated by mini pump and were renewed in every 3 d. After 4-wk of the sufficient-Pi and 6-wk of deficient-Pi treatments, the transgenic and WT plants were subjected to the assessment of the plant phenotypic features, drymass, total P contents, photosynthetic parameters, and the antioxidant enzymatic activities. Of these, the plant phenotypic features were evaluated by digital camera images. Plant drymass and total P contents were measured as described by Sun *et al*. (2012). Photosynthetic parameters such as photosynthetic rate (P_n), PSII efficiency (φPSII), and nonphotochemical quenching (NPQ) were determined as described by Guo *et al*. (2013). Antioxidant enzymatic activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and contents of malondialdehyde (MDA), a biomarker reflecting the cellular damage degree by reactive oxygen species (ROS), were assayed as described by Li *et al*. (2014a).

Five tobacco phosphate transporter (PT) genes currently released in GenBank were subjected to detection of the expression in both the Pi-deprived transgenic and WT seedlings to understand whether they were regulated by *TaMADS51* and involved in mediating plant Pi acquisition under Pi deprivation. These PT genes included *NtPT* (DI040486), *NtPT1* (AF156696), *NtPT2* (AB042950), *NtPT3* (AB042951), and *NtPT4* (AB042956). Root samples of the lines 3 and 8 as well as WT were used as the templates in qPCR analysis. Primers to amplify these PT genes are lised in Appendix B.

2.8. Statistical analysis

Averages of expression levels of the differentially expressed MADS genes, drymass, total P contents, accumulative P amount per plant, and photosynthetic parameters were derived from triplicate results. Standard errors of the averages were calculated and analyzed by using the Statistical Analysis System software (SAS Corporation, Cory, NC, USA).

3. Results

3.1. Molecular characterization of the wheat MADS genes

Totally 54 probe sets corresponding to the nonredundant wheat MADS genes were identified. Based on annotations of the probe sets supplemented in the gene chip, 31 of them were found to be released in NCBI GenBank and another 23 to be tentative consensus (TCs) stored in DFCI database. The names, GenBank accession numbers, and the probe set identities of these wheat MADS genes are listed in Appendix A.

The GenBank released MADS genes share cDNA lengths from 683 (*TaMADAGL40*) to 1 297 bp (*TaMADAGL11*) and encode polypeptides from 170 (*TaMADWLHS1-A*) to 274 aa (*TaMADAGL29*) that possess MWs of 19.21 (*TaMADWLHS1-A*) to 31.33 kDa (*TaMADAGL29*) and isoelectric points (pIs) of 5.74 (*TaMADAGL11*) to 9.63 (*TaMADAGL7*) (Appendix A). All of the translated proteins harbor the conserevd DNA binding domain and the MAD box that both locate at the N terminus, with appoximately 38-aa and 77-aa in length, respectively (Appendix C). Phylogenetic analysis revealed that the wheat MADS genes are categoried into four subgroups (I–IV) in which contain members of 11, 5, 10, and 4, respectively (Fig. 1), suggesting that they have evolved from different evolutional pathways.

3.2. Eexpression patterns of the wheat MADS genes under Pi sufficiency

Microarray analyses were performed to determine the expression patterns of the 54 wheat MADS genes under the sufficient-Pi condition. Dramatic variations were observed in the expresson levels among the MADS genes. Based on the intensity reads that represent expression levels, these MADS genes were categoried into various expression groups, including those of high, medium, low, and very low. The expression groups of high, medium, low, and very low contained the gene numbers of 6, 14, 25, and 9, respectively (Table 1). These results indicate that the wheat MADS genes are differently transcriptional regulated

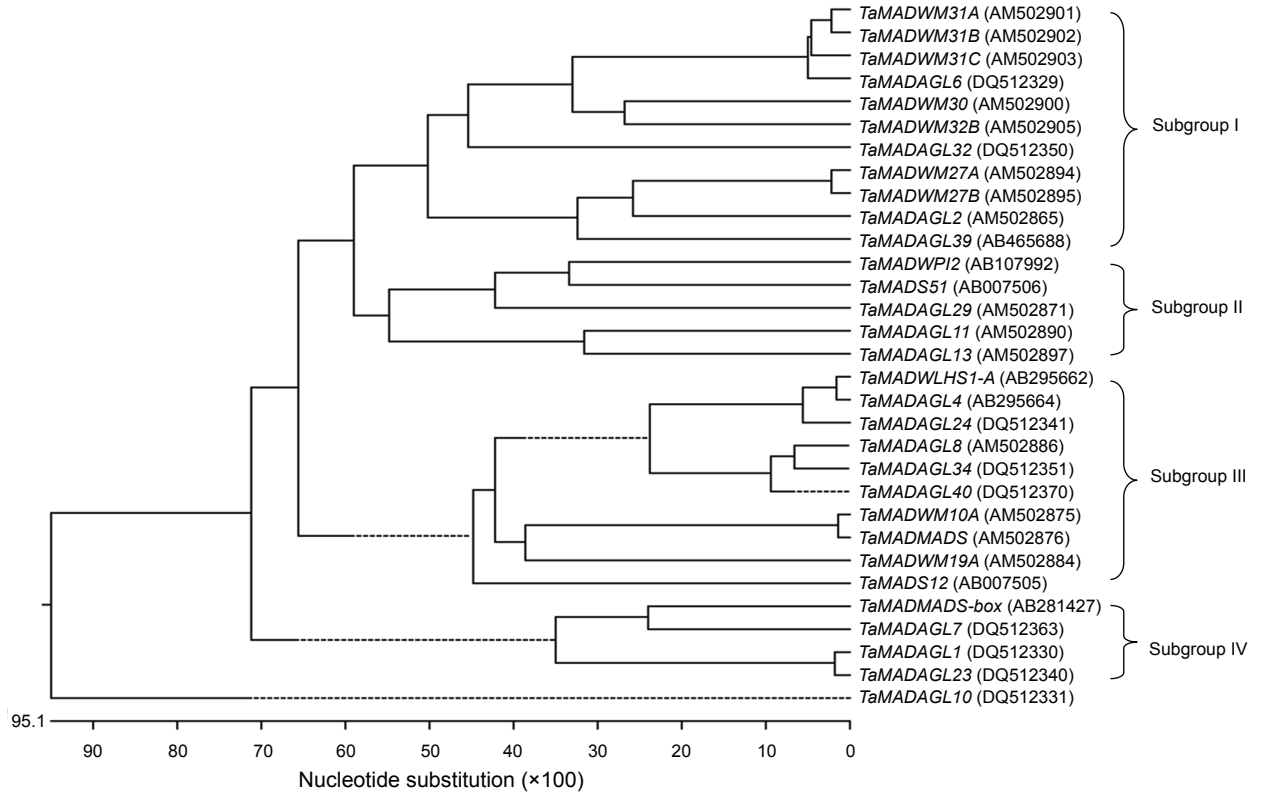

Fig. 1 Phylogenetic tree of the wheat MADS TF genes.

by Pi sufficiency.

3.3. Responses of the wheat MADS genes to Pi deprivation

Once exposed to Pi deprivation, most of the wheat MADS genes altered their expression levels (Table 1), suggesting that a large set of wheat MADS genes are regulated by the Pi-starvation sagnaling. Among them, nine genes were shown to be differentially expressed under Pi deprivation, including five (*TaMADS51*, *TaMAD;4*, *TaMAD;5*, *TaMAD;6*, *TaMAD;18*) to be upregulated and four (*TaMADAGL2*, *TaMADWM31C*, *TaMADMW32B*, *TaMAD;14*) to be down-regulated. These significantly up- and down-regulated MADS genes together with their $\log_2$ ratios on intensity reads between those under Pi deprivation (1, 6, and 24 h) and those under Pi sufficiency (0 h) are listed in Table 2. These results suggested that distinct MADS genes are possibly involved in mediating plant responses or tolerance to the low-Pi stress.

3.4. Temporal and spatial expression patterns of the differentially expressed MADS genes

The temporal and spatial expression patterns in roots, leaves and young spikes of several differentially up- and down-regulated MADS genes were investigated under the contrasting Pi-supply conditions. In accordance with the results of aforementioned microarray analysis, the expression levels of *TaMADS51*, a sigificantly upregulated MADS gene in the microarray analyses, exhibited more transcripts in various tissues at different growth stages under Pi deprivation compared with those under Pi sufficiency (Fig. 2-A). By contrast, the expressions of *TaMADAGL2*, *TaMADWM31C* and *TaMADWM32B*, three differentially downregulated genes by Pi deprivation, were significantly repressed in roots, leaves and young spikes at the assayed growth stages under Pi deprivation compared with those under Pi sufficiency (Fig. 2-B). These results indicate that the microarray analysis results are credible and reproducible.

3.5. Plant growth characterization of the *TaMADS51*-overexpressing plants under Pi deprivation

A phylogenetic tree covering *TaMADS51* and its homologous partners was generated and is shown in Appendix D. *TaMADS51* shares high identities with *TaMADS82* (AB107993), *TaMADWM13* (AM502879), *Hordeum vulgare* cDNA (AK373398), *HvAPETALA3-like* (AY541065), and *BdMADS16* (NM_001302871) (with similarities of 88.3

Table 1 Expression intensities of the wheat MADS genes under the sufficient- and deficient-phosphate (Pi) conditions

Expression group under Pi sufficiency	Gene name	Accession no.	Normalized intensity reads[1)]			
			0 h (Pi sufficiency)	1 h of Pi deprivation	6 h of Pi deprivation	24 h of Pi deprivation
High (6)	*TaMADAGL1*	DQ512330	2 568.38	3 049.64	3 255.29	3 258.87
	TaMADAGL13	AM502897	1 945.61	1 552.04	1 456.96	1 694.50
	TaMADAGL23	DQ512340	1 513.25	1 986.72	2 027.26	1 873.05
	TaMAD;3	TC372780	1 367.12	2 647.02	2 472.35	2 302.84
	TaMAD;15	TC404326	1 143.10	825.98	1 016.23	825.63
	TaMAD;21	TC446981	1 449.33	1 094.99	797.62	1 039.05
Medium (14)	*TaMADAGL4*	AB295664	830.27	1 084.11	981.14	740.03
	TaMADAGL7	DQ512363	154.23	209.04	200.14	189.10
	TaMADAGL11	AM502890	101.49	90.636	88.54	92.00
	TaMADAGL24	DQ512341	379.97	476.58	421.39	376.62
	TaMADAGL32	DQ512350	421.76	661.11	348.29	659.45
	TaMADMADS	AM502876	201.97	222.88	261.41	217.47
	TaMADS51	AB007506	177.27	561.19	604.19	516.94
	TaMADWPI2	AB107992	114.42	115.53	186.72	135.16
	TaMADWLHS1-A	AB295662	580.93	713.07	663.32	487.79
	TaMADWM32B	AM502905	755.57	381.29	248.16	293.15
	TaMAD;2	TC420046	127.02	109.05	116.25	138.53
	TaMAD;4	TC445232	348.83	1 714.33	1 205.77	770.68
	TaMAD;5	TC393933	527.19	1 376.18	939.10	716.39
	TaMAD;16	TC404376	688.60	868.22	987.29	727.52
Low (25)	*TaMADAGL2*	AM502865	50.48	21.50	16.06	23.30
	TaMADAGL6	DQ512329	86.91	55.41	47.27	59.25
	TaMADAGL8	AM502886	50.22	44.85	46.50	50.39
	TaMADAGL10	DQ512331	26.78	21.31	37.67	28.82
	TaMADAGL34	DQ512351	22.45	18.66	32.56	12.54
	TaMADWM27A	AM502894	14.61	12.711	15.91	9.73
	TaMADWM27B	AM502895	13.07	12.24	12.34	14.74
	TaMADWM30	AM502900	80.44	64.01	71.64	74.54
	TaMADWM31A	AM502901	35.57	23.35	33.37	25.60
	TaMADWM31B	AM502902	86.68	64.58	45.10	55.23
	TaMADWM31C	AM502903	13.54	6.22	6.58	7.15
	TaMADS-box	AB281427	85.91	105.78	90.73	100.01
	TaMADS12	AB007505	13.10	10.48	8.48	15.69
	TaMAD;6	TC399203	71.72	182.65	225.88	166.34
	TaMAD;7	TC410384	18.65	33.02	32.14	16.56
	TaMAD;8	TC417485	19.18	26.14	47.63	28.21
	TaMAD;9	TC459187	82.92	122.82	264.9	150.77
	TaMAD;10	TC391482	87.28	97.07	119.14	100.37
	TaMAD;12	TC371052	56.86	76.35	84.79	79.43
	TaMAD;14	TC393565	18.25	7.72	5.18	8.6
	TaMAD;17	TC409709	65.17	42.49	60.46	56.84
	TaMAD;18	TC412868	30.93	110.39	69.12	79.32
	TaMAD;20	TC433067	16.01	8.63	19.09	9.99
	TaMAD;22	DY761369	22.39	38.99	24.2	36.31
	TaMAD;23	TC425482	10.15	4.95	9.45	7.98
Very low (9)	*TaMADAGL29*	AM502871	7.96	4.88	5.08	7.78
	TaMADAGL39	AB465688	8.80	6.23	14.44	13.27
	TaMADAGL40	DQ512370	7.73	6.92	9.43	8.44
	TaMADWM10A	AM502875	2.96	4.28	7.29	5.48
	TaMADWM19A	AM502884	6.76	6.67	10.02	22.98
	TaMAD;1	TC374784	1.84	1.61	1.22	5.72
	TaMAD;11	TC378148	4.47	6.40	11.56	17.14
	TaMAD;13	TC375063	7.20	5.26	17.26	10.01
	TaMAD;19	TC415008	4.27	2.77	6.55	2.29

[1)] 1, 6 and 24 h stand for the time points under Pi deprivation and 0 h stands for the time point under Pi sufficiency.

Table 2 Log_2 ratios of the transcript abundance of the differentially expressed MADS genes under Pi deficiency to that under Pi sufficiency

Gene name	Accession no.	log_2 ratio		
		1 h/0 h	6 h/0 h	24 h/0 h
TaMADS51	AB007506	1.66	1.77	1.54
TaMAD;4	TC445232	2.30	1.79	1.14
TaMAD;5	TC393933	1.38	0.83	0.44
TaMAD;6	TC399203	1.35	1.66	1.21
TaMAD;18	TC412868	1.84	1.16	1.36
TaMADAGL2	AM502865	–1.23	–1.65	–1.12
TaMADWM31C	AM502903	–1.12	–1.04	–0.92
TaMADWM32B	AM502905	–0.99	–1.61	–1.37
TaMAD;14	TC393565	–1.24	–1.82	–1.09

to 95.3% at the nucleic acid level), suggesting that it has evolved from similar ancestors with its above homologs.

Nine transgenic tobacco lines trasnsformed by *TaMADS51* at T3 generation together with WT were subjected to the expression analysis. All of the transgenic lines exhibited strong *TaMADS51* expression, in which lines 3 and 8 were much higher than others (Fig. 3-A). *TaMADS51* was also detected in WT (Fig. 3-A), indicating that the tobacco genome also harbors a *TaMADS51* homolog although it has not been released in the GenBank.

The transgenic lines 3 and 8 together with WT were subjected to investigation of the *TaMADS51* function in mediating plant tolerance to the Pi-starvation stress. Southern blot analysis revealed that the two transgenic lines both harbored one copy of the target gene (Appendix E). Under Pi sufficiency, the growth features, plant drymass, total P contents, and plant accumulative P were similar to each other between the transgenic lines and WT (Figs. 3-B and 4-A–C). Similarly, no obvious variations were observed in the photosynthetic parameters of P_n, φPSII and NPQ, and the antioxidant enzymatic activities of SOD, CAT and POD as well as the contents of MDA between the transgenic and

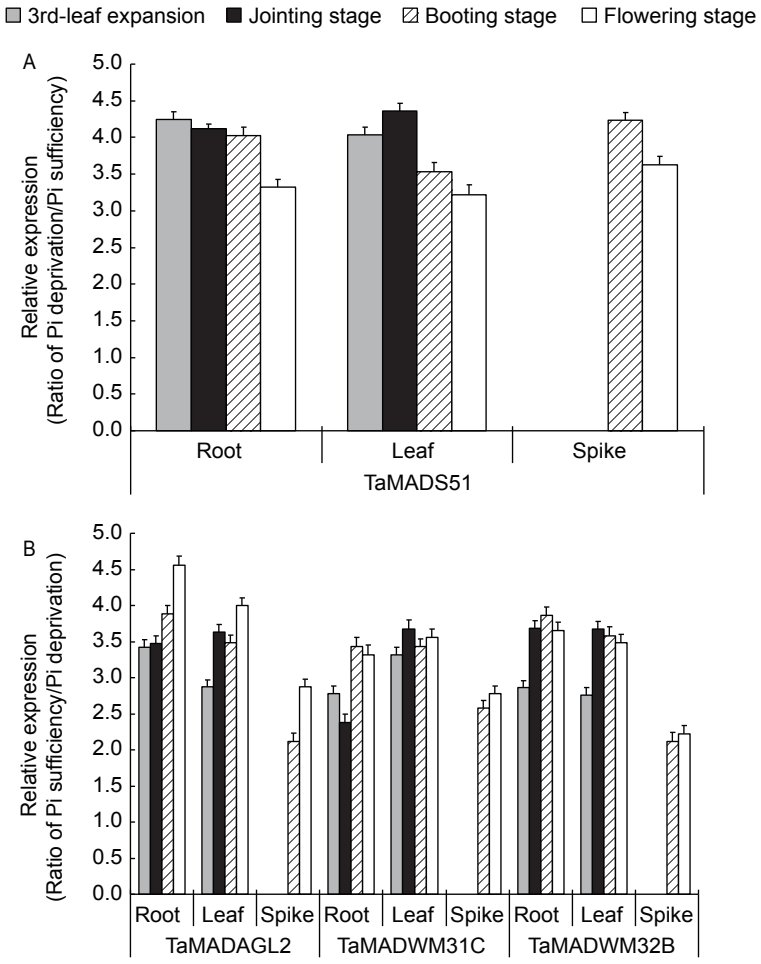

Fig. 2 Expression levels in roots, leaves and young spikes of the differentially expressed genes at various growth stages detected by qPCR. A, differentially up-regulated expressed gene. B, differentially down-regulated expressed genes. Data are shown ratios compared with internal standard, which is set to 1. Bars are SE. The same as below.

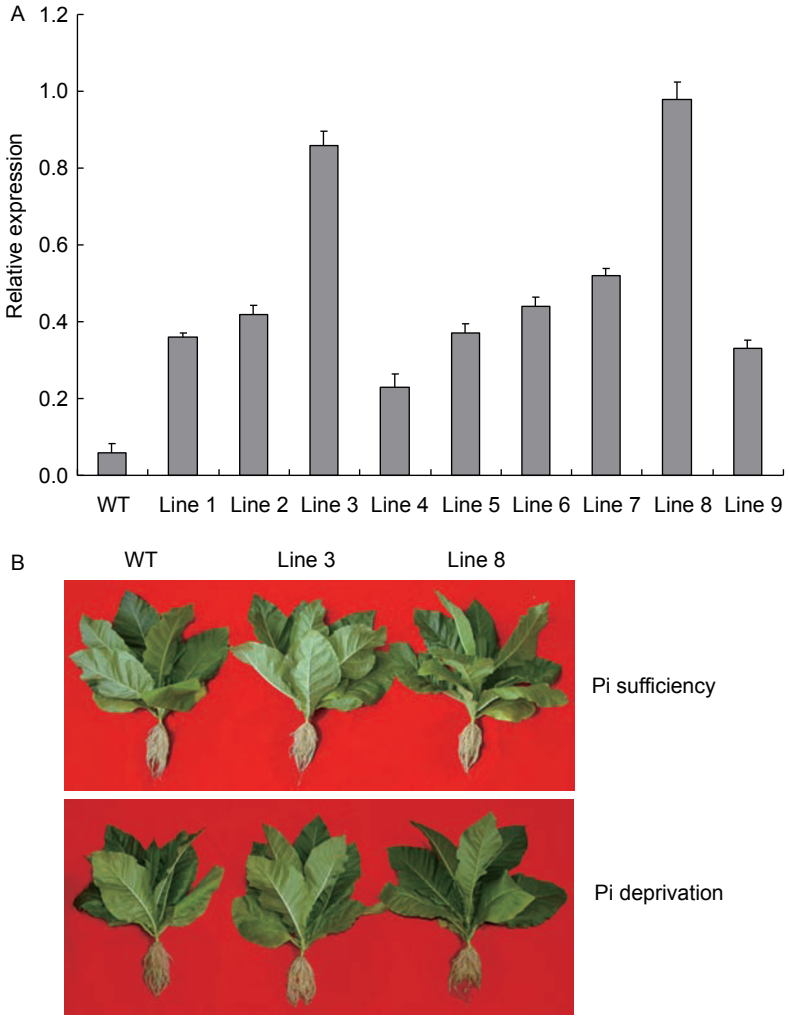

Fig. 3 Molecular characterization of the transgenic tobacco plants with overexpression of *TaMADS51*. A, expression levels of *TaMADS51* in the phosphate (Pi)-deprived transgenic and wild type (WT) plants detected by qPCR. B, growth features of the transgenic lines 3 and 8 as well as WT under Pi sufficiency and Pi deprivation. Lines 1 to 9, nine transgenic lines with integration of *TaMADS51*. Lines 3 and 8, two transgenic lines with strong expression of *TaMADS51*.

WT plants (Figs. 5-A–C and 6-A–D). In contrast, under Pi deprivation, the transgenic lines exhibited larger phenotypic features, more plant drymass and accumulative P amount per plant (Figs. 3-B and 4-A, C), and higher P_n, φPSII, and lower NPQ (Fig. 5-A–C), higher activities of SOD, CAT and POD as well as lower contents of MDA than WT (Fig. 6-A–D). Among them, the transgenic lines displayed increased values on accumulative P amount, P_n, φPSII, SOD, CAT, and POD with 16.4–31.6, 10.3–20.0, 22.0–28.4, 14.8–20.3, 23.7–30.7, and 12.2–22.4% as well as decreased values on NPQ and MDA with 10.6–18.8 and 22.6–33.5%, respectively, relative to the WT. Although the transgenic lines displayed significantly more drymass and plant accumulative P, the total P contents were similar between the transgenic and WT plants under Pi deprivation (Fig. 4-B). These results indicate that overexpression of *TaMADS51* can significantly improve the plant growth and P accumulation in plants under Pi deprivation.

To determine whether the improved plant P accumulation in the transgenic lines are associated with distinct phosphate transporter (PT) genes regulated by *TaMADS51*, the expressions of *NtPT* (DI040486), *NtPT1* (AF156696), *NtPT2* (AB042950), *NtPT3* (AB042951), and *NtPT4* (AB042956), five phosphate transporter encoding genes in tobacco, were subjected to expression analysis in the transgenic and WT plants under Pi deprivation. Although the expression levels varied among the PT genes, no variations in the expression intensity were detected between the transgenic and WT plants for all of the tested genes (Appendix F). Thus, the altered plant P accumulation between the transgenic and WT plants under Pi deprivation were possibly attributed to variations of the root archetecture system in which the

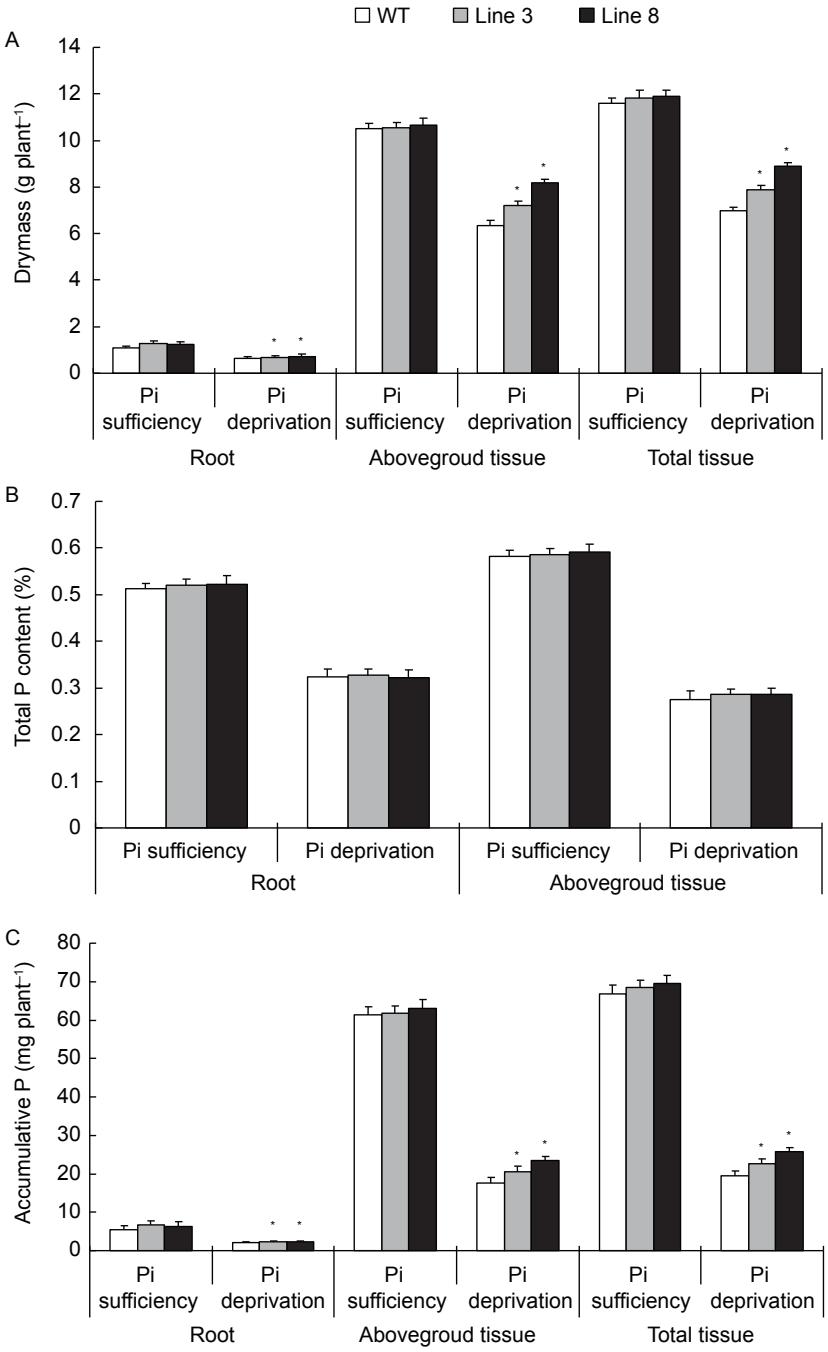

Fig. 4 Drymass, total P contents and accumulative P amount in the transgenic and WT plants under different Pi-supply conditions. A, drymass. B, total P contents. C, plant accumulative P amount. * represents to be significant compared with WT (P<0.05). The same as below.

transgenic plants possessed higher Pi acquisition efficiency than the WT plants.

4. Discussion

Transcription factors (TFs) are essential regulators of gene transcription through their two functional domains, namely, the DNA-binding domain and the activation/repression domain (Du *et al*. 2012; Li *et al*. 2014b). In this study, 31 of the wheat MADS TF genes released in GenBank were subjected to the molecular characterization analysis. Phylogenetic analysis categoried these MADS genes into four subgroups that contain varied numbers. These results suggest that the wheat MADS genes have possibly evolved from different

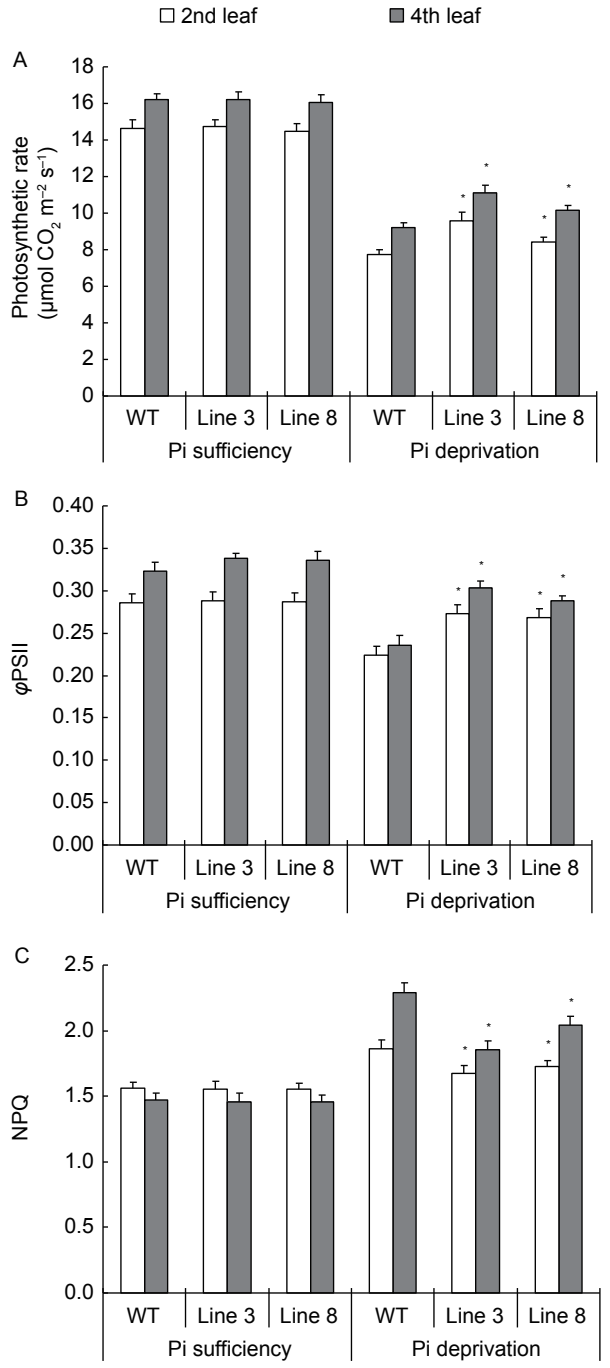

Fig. 5 Photosynthetic rate (P_n, A), PSII efficiency (φPSII, B), and nonphotochemical quenching (NPQ, C) in the transgenic and WT plants under different Pi-supply conditions.

ansestors which extended gradually to the larger subgroups by duplication events along with the evolutionary process. The conserved MADS box and the DNA binding domain in these MADS proteins indicate that they exert distinct biological roles in plants.

MADS TF genes have been extensively associated with the plant growth and developmental processes in diverse plant species (Yoshida and Nagato 2011; Smaczniak *et al*. 2012). Recently, plant MADS transcription factors have also been shown to involve the plant responses and adaptation to the biotic and environmental stresses (Misson *et al*. 2005; Gupta *et al*. 2012). In this study, based on microarray analyses, nine of the wheat MADS genes were identified to be differentially regulated by Pi deprivation, including five to be significantly upregulated and four to be significantly downregulated. Based on qPCR analyses, we confirmed that part of them, including the one of upregulated gene (*TaMADS51*) and three of downregulated genes (*TaMADAGL2*, *TaMADWM31C* and *TaMADWM32B*), exhibited similar expression patterns temporally and spatially in various tissues such as in roots, leaves and young spikes, to those detected by the microarray analyses. Therefore, these differentially expressed MADS genes are suggested to involve in mediating the Pi-starvation signaling transduction and function in regulating plant responses or tolerance to the low-Pi stress.

Distinct *cis*-acting regulatory elements situated in the promoter region are confirmed to be crucial in controlling the gene transcriptional variations upon Pi deprivation. For instance, *P1BS* and *PIBS*-like, two *cis*-acting regulatory motifs, play an important role in enhancing the transcription of Pi-starvation responsive genes (Glassop *et al*. 2005). Mutations of the base in *P1BS* element of the Pi-starvation responsive genes cause drastically impairment in gene response to Pi deprivation and alter largely the morphology of root and the internal metabolic responses (Rubio *et al*. 2001). Thus, characterization of the differentially expressed MADS gene promoters can dissect the roles of *PIBS* and *PIBS*-like elements as well as other novel low-Pi responsive motifs on which to provide new insights into understanding their transcription mechanisms in responding to the varied external Pi signaling.

The function of *TaMADS51* in mediating plant tolerance to the Pi-starvation stress was investigated through gene transformation approach in this study. In comparison with WT, lines 3 and 8, two transgenic lines with strong *TaMADS51* expression, exhibited improved plant growth features, plant drymass and accumulative P amount, photosynthetic parameters, and cellular ROS homeostasis under the deficient-Pi condition. These results confirm that *TaMADS51* acts as one of critical regulators in improving plant tolerance to Pi deprivation. Pi acquisitions in roots are largely mediated by phosphate transporters (PTs) that are located in the cytoplasmic membranes of epidermal cells and root hair (Nussaume *et al*. 2011). PT encoding genes have currently been functionally characterized in *Arabidopsis*, rice and oth-

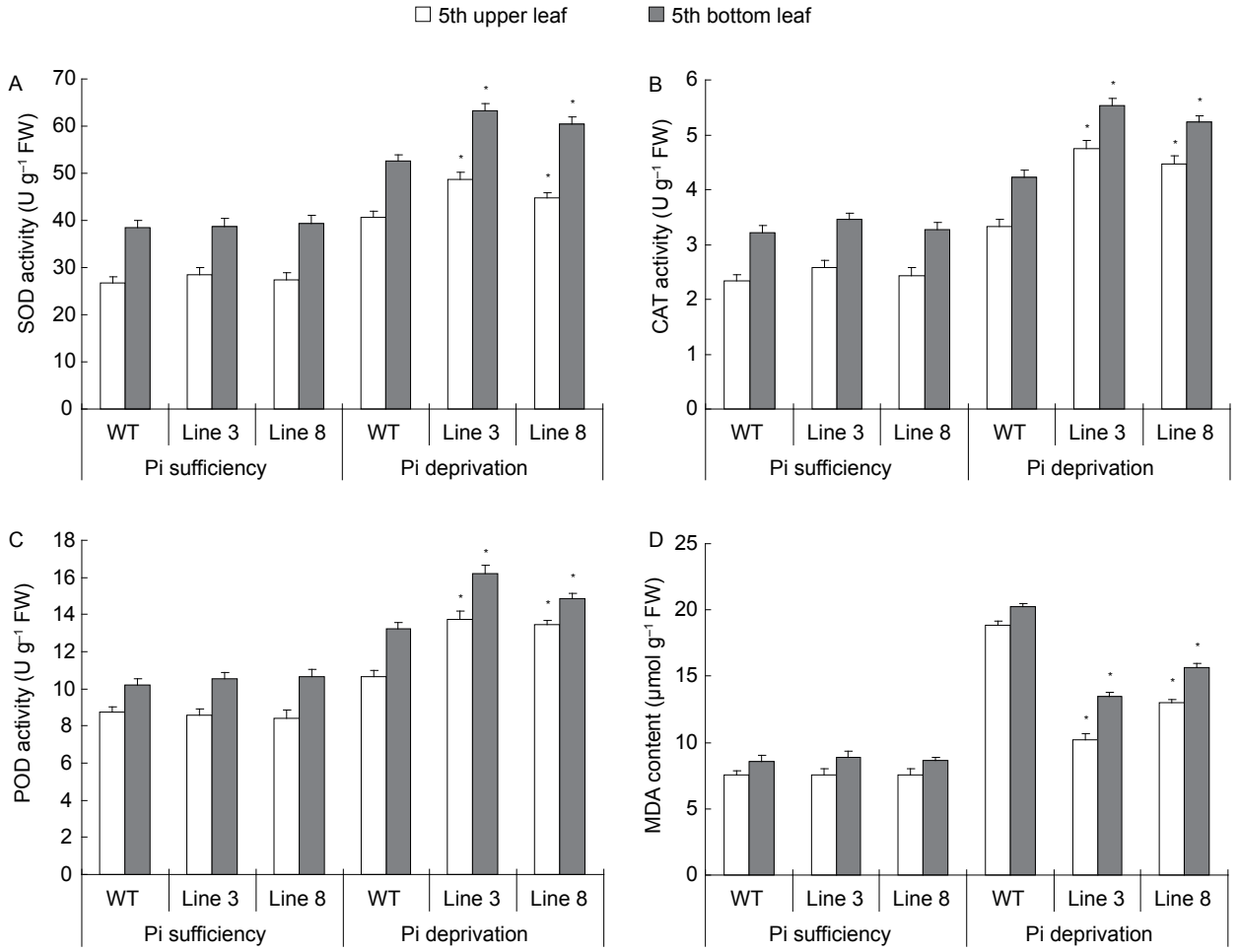

Fig. 6 Enzymatic activities of superoxide dismutase (SOD, A), catalase (CAT, B), peroxidase (POD, C), and the contents of malondialdehyde (MDA, D) in the transgenic and WT plants under different Pi-supply conditions.

er plant species, confirming that part of them are important in regulating Pi acquisition under the Pi-deprived conditions (Guo *et al*. 2013; Liu *et al*. 2013). To address weather the PT genes are transcriptional regulated by *TaMADS51* on which to improve the plant P nutrition under Pi deprivation, five tobacco PT encoding genes, including *NtPT*, *NtPT1*, *NtPT2*, *NtPT3*, and *NtPT4* released in the GenBank, were subjected to expression analysis in the transgenic and WT plants under the Pi-starvation condition. None of these genes exhibited altered expression levels in the transgenic plants relative to WT. These results suggest that the improvement of the plant P accumulation under Pi deprivation regulated by *TaMADS51* is possibly attributed to the root architecture system that increased the effective surface areas for Pi acquisition. However, it can not be ruled out the posibility that other PT genes regulated by *TaMADS51* currently uncharacterized in tobacco are functional in mediating plant Pi acquisition in the transgenic plants. Further investigation of the transcriptome regulated by *TaMADS51* will be helpful to dissect the gene network that *TaMADS51* mediates plant tolerance to the Pi-starvation stress.

5. Conclusion

The wheat MADS genes share the cDNA full lengths of 683 to 1 297 bp that encode the amino acids of 170 to 274 aa. Under Pi sufficiency, the MADS genes showed drastically varied transcripts and they were categoried into different expression groups based on the expression levels. Five of these MADS gene were upregulated (*TaMADS51*, *TaMADS4*, *TaMADS5*, *TaMADS6*, and *TaMADS18*) and four of them were downregulated (*TaMADAGL2*, *TaMADWM31C*, *TaMADWM32B*, and *TaMADS;14*) under Pi deprivation. Overexpressing *TaMADS51* in tobocco greatly improved the plant growth features, drymass, Pi acquisitions, and photosynthetic parameters as well as the antioxidant enzymatic activities under the Pi-deficient treatment. Our investigation herein indicates that distinct wheat MADS

genes are transcriptional response to Pi deprivation and play critical roles in mediating plant tolerance to the Pi-starvation stress through thanscriptional regulation of the downstream Pi-responsive genes.

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Appendix associated with this paper can be available on http://www.ChinaAgriSci.com/V2/En/appendix.htm

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